to determine whether C is part of the mechanism by which A contributes to B. Biologists use convergency and consistency amongst these experiments to judge the strength of any causal assertion. Therefore, in research maps convergency and consistency amongst results increases the score assigned to each edge, while contradictory results have the opposite effect. By selecting any one edge in a particular research map, users can be directed to the exact experiments and associated research papers represented by that edge. Of course, there is much more to researchmaps, but this gives you a sense of what these maps are all about.

***Is this the theme of your recent book* Engineering the Next Revolution in Neuroscience?** I smile every time I read that title… It was intentionally provocative. Nevertheless, the problem we discuss in the book, the problem we tackle in our researchmaps project, is a big problem, and solving it will undoubtedly 'revolutionize' not only neuroscience, but perhaps any other field of science where causal information is key to progress. I know that this is a big claim, but it never pays to be shy about big problems. Beyond big data problems that individual neuroscientists face, the book also discusses the need for formal studies of how to optimize research planning. I dream of a time when scientific choice will be as rigorous and principle-based as algebra and geometry. This by no means excludes human creativity from the scientific process! No, it simply hones our creativity, focuses it onto those areas where it will be most useful and productive. The book really is about these and related themes. By the way: if we are ever to know how we learn and transform the world around us, we need formal tools like researchmaps to get there, and we may even need them to recognize that we have arrived.

Integrative Center for Learning and Memory, Department of Neurobiology, Department of Psychology, Department of Psychiatry, Brain Research Institute, University of California, Los Angeles, CA 90095 USA.
E-mail: silvaa@mednet.ucla.edu, silvaa@ucla.edu, alcinojsilva@gmail.com

## Quick guide

# Chromothripsis

Emily M. Hatch and Martin W. Hetzer*

***What is chromothripsis?*** The word 'chromothripsis' literally means 'chromosome shattering'. Chromosomes that undergo chromothripsis first fragment into many pieces and then get stitched back together in a random order by DNA repair processes, most likely non-homologous end joining. This generates a highly rearranged chromosome from a single catastrophic event (Figure 1). Previous complex chromosome rearrangements could be explained by multiple independent breakage and repair events accumulating on a chromosome over time. However, specific features of chromothripsis sequences — including highly clustered breakpoints, no segment amplification, and alternating retention and loss of heterozygosity along the chromosome — make it likely that the chromosome is breaking all at once. Currently, chromothripsis has been identified in cancer cells and in the male germline.

***Why haven't I heard about this before?*** Chromothripsis was discovered fairly recently by paired-end sequencing in chronic lymphocytic leukemia. A mixture of whole genome sequencing, array-based comparative genomic hybridization (aCGH), and single nucleotide polymorphism (SNP) array analyses have now identified these massive chromosome rearrangements in many types of cancer. These data uncovered an unanticipated amount of small chromosome rearrangements and renewed interest in how chromosome structural variation contributes to cancer development.

Several types of 'all-at-once' chromosome rearrangement processes, including chromothripsis, have now been described. Chromoanasynthesis looks very similar to chromothripsis in that it often affects a single chromosome or chromosome arm, but is characterized by the amplification of numerous segments and has signatures of replication-mediated repair. Chromoplexy occurs when multiple DNA breaks are present throughout the genome at one time and each end of each break finds a different partner to pair with during DNA repair. This results in the joining of many distant loci and chromosomes together. The term 'chromoanagenesis' (chromosome rebirth) has been proposed to describe these new types of complex chromosome rearrangements.

***How bad is chromothripsis?*** In tumor cells chromothripsis has been shown to result in the loss of tumor suppressors and dysregulation of genes with known cancer links. In addition, shattering can cause oncogene amplification. Chromosome segments that fail to get reincorporated into the main chromosome can circularize to become double minutes. These small DNA circles are frequently amplified and, if oncogenes such as *MYC* are present within the double minutes, they become massively upregulated. Because chromothripsis affects a large number of genes at once, it can bypass the time delay inherent in the gradual accumulation of mutations and quickly stimulate cancer development or evolution. Consistent with this, chromothripsis is associated with poor prognosis in several cancer types (e.g. neuroblastoma), although it is unclear whether this is a causal link.

The flip side of affecting a large number of genes at once is that most chromothripsis events are going to be lethal. Significant misregulation of gene expression, loss of heterozygosity, and increased aneuploidy as a result of segments being lost are likely to be detrimental to the cell, regardless of which particular sequences are hit. This point is clear from examples of germline chromothripsis. Patients with congenital disease due to chromothripsis typically have few rearrangements and have retained almost all of the chromosome pieces. In cancer, chromothripsis has been correlated with loss of pathways that stabilize genome stability, such as inactivation of p53. In addition, cancer cells often undergo changes, such as an increase in ploidy, that can buffer the deleterious effects of aneuploidy and thus could generate an environment in which the benefits of highly rearranged chromosomes can outweigh the negative consequences.

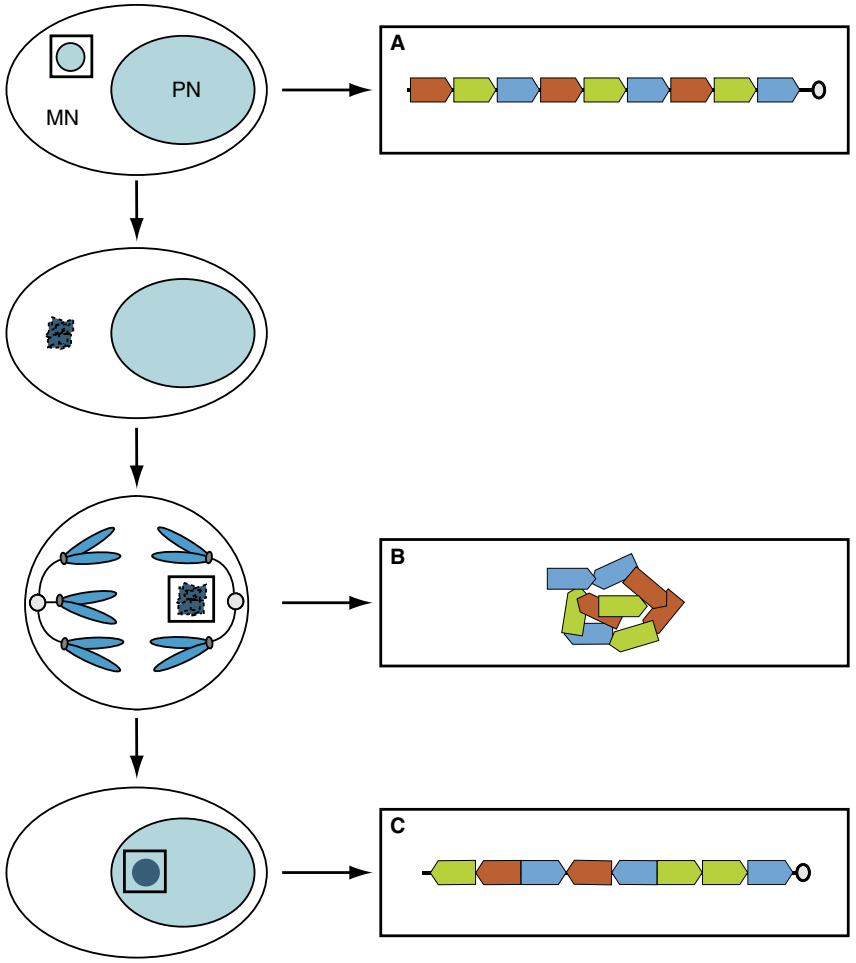


**Figure 1. Model of the micronucleation mechanism of chromothripsis.**
When a chromosome missegregates during mitosis in mammalian cells and forms a micronucleus (MN) (A), it can undergo extensive DNA fragmentation as a result of nuclear envelope disruption in interphase (B). This damaged chromosome can segregate with the rest of the chromatin and end in up in the primary nucleus (PN) after the next mitosis. At that point it can undergo non-homologous end joining to generate a highly rearranged chromosome in which some sequences have been lost (C).

***What possible mechanism could restrict these massive rearrangements to a single chromosome?*** In chromothripsis, breaks can be localized to a single chromosome or chromosome region. Many different chromosomes have been found to be rearranged by chromothripsis in different cancer types, suggesting that the mechanism of chromosome shattering is not defined by chromosome identity. In addition, no DNA damage mechanism is known that could target a single allele of a single chromosome in the context of the nucleus. Although many factors likely contribute to chromothripsis, one model has emerged that explains the localized nature of the DNA damage.

This model is based on the well-known observation that individual chromosomes in mammalian cells can form their own nuclear compartments, called micronuclei. These micronuclei form from errors in mitosis that cause a whole chromosome, or a fragment of a broken one, to lag behind the rest of the chromosomes at anaphase or fail to align on the spindle. Micronuclei are very common in cancer cells and have been widely used as markers of chromosome instability. Their frequency in healthy tissue is unclear, but in cultured fibroblasts a micronucleus is formed in about 1 out of every 100 divisions.

It had been previously noticed that micronuclei could accumulate a massive amount of DNA damage during interphase and then appear fragmented in mitosis. These observations led to the current hypothesis that chromothripsis results from a micronucleated chromosome being shattered in one cell cycle and then randomly stitched back together in the next. In this mechanism only one chromosome is broken up and available for end joining in G1 and thus rearrangements are limited. However, if additional breaks were present during DNA repair, or if more than one chromosome was in the micronucleus, multiple chromosomes could be involved.

The nuclear envelope in primary nuclei normally remains intact throughout interphase. However, in micronuclei the membrane is much more fragile and has a high probability of rupture, causing a loss of compartmentalization. In addition, micronuclei have problems replicating their DNA in a timely manner. When micronuclear rupture occurs in the presence of replicating DNA, the hypothesis is that it causes the simultaneous collapse of replication forks and results in widespread DNA damage. Because micronuclei do not repair their nuclear envelope, they cannot accumulate DNA repair proteins and the damaged chromatin persists into mitosis. Micronucleation is not the only possible mechanism of chromothripsis, however, and several other models have been proposed to explain its origin, including aborted apoptosis and telomere dysfunction. At this time, it is unclear which will be the most prevalent.

***How much of an impact is chromothripsis going to have on our understanding of disease?*** It is currently clear that chromothripsis can provide a selective advantage to cancer cells in certain conditions and it has been connected to congenital diseases in multiple patients. However, a causative link between chromothripsis and these diseases is still lacking, and many questions remain about the process. First, the overall frequency of chromothripsis in germline cells, healthy tissue, and cancer types is unknown; it is likely that the identified examples represent only the small proportion of cells that have undergone this event and have survived. Second, genomic analysis of tumors suggests that the frequency of chromothripsis differs

 

CellPress

dramatically between cancer types. However, the question of whether these differences represent changes in the frequency of chromothripsis between different cell types or changes in the ability of different cell types to survive chromothripsis remains unclear. Finally, additional work will be required to identify the time at which chromothripsis occurs. The micronucleus model of chromothripsis suggests that complex chromosome rearrangements could occur in healthy cells, and evidence of chromothripsis in primary tumor samples suggests that it can be an early event in cancer development. However, whether chromothripsis is important for early cancer development or continued evolution of a tumor after it is formed remains to be seen.

### *Where can I find out more?*

Crasta, K., Ganem, N.J., Dagher, R., Lantermann, A.B., Ivanova, E.V., Pan, Y., Nezi, L., Protopopov, A., Chowdhury, D., and Pellman, D. (2012). DNA breaks and chromosome pulverization from errors in mitosis. Nature *482*, 53–58.

Hatch, E.M., Fischer, A.H., Deerinck, T.J., and Hetzer, M.W. (2013). Catastrophic nuclear envelope collapse in cancer cell micronuclei. Cell *154*, 47–60.

Holland, A.J., and Cleveland, D.W. (2012). Chromoanagenesis and cancer: mechanisms and consequences of localized, complex chromosomal rearrangements. Nat. Med. *18*, 1630–1638.

Kloosterman, W.P., and Cuppen, E. (2013). Chromothripsis in congenital disorders and cancer: similarities and differences. Curr. Opin. Cell Biol. *25*, 341–348.

Kloosterman, W.P., Guryev, V., van Roosmalen, M., Duran, K.J., de Bruijn, E., Bakker, S.C.M., Letteboer, T., van Nesselrooij, B., Hochstenbach, R., Poot, M., *et al*. (2011). Chromothripsis as a mechanism driving complex de novo structural rearrangements in the germline. Hum. Mol. Genet. *20*, 1916–1924.

Korbel, J.O., and Campbell, P.J. (2013). Criteria for inference of chromothripsis in cancer genomes. Cell *152*, 1226–1236.

Rausch, T., Jones, D.T.W., Zapatka, M., Stutz, A.M., Zichner, T., Weischenfeldt, J., Jager, N., Remke, M., Shih, D., Northcott, P.A., *et al*. (2012). Genome sequencing of pediatric medulloblastoma links catastrophic DNA rearrangements with TP53 mutations. Cell *148*, 59–71.

Stephens, P.J., Greenman, C.D., Fu, B., Yang, F., Bignell, G.R., Mudie, L.J., Pleasance, E.D., Lau, K.W., Beare, D., Stebbings, L.A., *et al*. (2011). Massive genomic rearrangement acquired in a single catastrophic event during cancer development. Cell *144*, 27–40.

Zhang, C.-Z., Leibowitz, M.L., and Pellman, D. (2013). Chromothripsis and beyond: rapid genome evolution from complex chromosomal rearrangements. Genes Dev. *27*, 2513–2530.

Molecular and Cell Biology Laboratory, Salk Institute for Biological Studies, La Jolla, CA 92037, USA.
*E-mail: hetzer@salk.edu

## Primer

# Bat flight

Anders Hedenström
and L. Christoffer Johansson

Bats are unique among extant flying animals, as they have compliant wings and an echolocation sensory system that distinguish them from birds and insects. Flying in the dark, guided by echolocation, has influenced the aerodynamics of bat flight perhaps more than previously realized and resulted in a characteristic flight that is now being revealed.

Bats evolved muscle-powered flight about 65 million years ago, alongside birds, pterosaurs (probably extinct when bats evolved) and insects. The oldest fossil bat dates 55 million years back and, hence, there is a 10 million year gap in the early evolution of bats where information about the initial adaptive radiation is still missing. The oldest well preserved bat fossils, *Onychronycteris finneyi* and *Icanonycteris index*, exhibit all of the features of modern bats, including elongated fingers to span out the wing surface and ear morphology suggesting that at least *Icanonycteris* was using echolocation. Since their earliest appearance, bats have diversified (Figure 1) and adapted to different ecological niches and many different flight strategies. The present count amounts to more than 1200 species, which means that one in five mammal species is a bat, only outnumbered by rodents. Their body size ranges from 2 g to 1.6 kg, a tenth of the size range of birds. Bat wings vary from short and broad in species that maneuver in cluttered habitats to long and narrow in species of the open airspace. Although the main wintering strategy in Northern hemisphere temperate climates is hibernation, some bats are migratory between summer reproductive areas and Southern wintering sites. Flight makes bats highly mobile and allows them to exploit many biomes and ecological niches. Here, we focus on the essential elements of bat flight.

## The bat airframe

There are many features that distinguish the bat airframe, the wings and body, from that of birds and insects. These features have consequences for their flight performance. The most apparent one is perhaps how the wing surface is built. In birds and insects, the wings are mainly constructed from dead material (keratin feathers or chitin cuticle) giving them a limited ability to actively control the wing surface shape. Bats, on the other hand, have a wing constructed from live skin stretched by the elongated arm and fingers. The skin is 4–10 times thinner than expected, and the bones have a reduced mineralization, compared to other similar sized mammals, reducing the weight of the wing considerably. Skin is living tissue, packed with sensors, elastic fibers and in the case of bat wings also with specialized muscles (Figure 2A). The skin is anisotropic, with higher compliance (i.e. being permissive to load) parallel to the trailing edge, affecting how the skin deforms when subjected to aerodynamic forces, as reflected in strain measurements during flight. Intrinsic muscles in the wing membrane (Figure 2A), not connected to any bones, are thought to control the stiffness of the membrane and thereby the wing's camber, the curvature of the wing profile. Recent studies have shown that these muscles are indeed active during specific phases of the wingbeat. Studies of artificial membranes with electrically controlled compliance have shown to be able to improve aerodynamic performance.

Having the wing stretched by fingers gives bats a high morphing ability, i.e. the ability to change the shape of the wing (Figure 1H). The fingers can spread and bend to different degrees, changing the wing area by stretching the membrane or controlling the camber of the wing and as a consequence the lift coefficient of the wing. (The lift coefficient is a measure of the efficacy of a wing to generate lift and is defined as $C_L = 2L/\rho U^2 S$, where $L$ is lift, $\rho$ is air density, $U$ is local speed about the wing and $S$ is wing surface area.) Recent studies of 3D kinematics of bats show that area and camber are indeed controlled throughout the wingbeat and across